ELSEVIER

Chemosphere 65 (2006) 889–897

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Mercury accumulation in grass and forb species as a function of atmospheric carbon dioxide concentrations and mercury exposures in air and soil

A.G. Millhollen [a], D. Obrist [a,b], M.S. Gustin [a,*]

[a] *Department of Natural Resources and Environmental Sciences, University of Nevada, Reno, NV 89557, USA*
[b] *Institute of Environmental Geosciences, University of Basel, 4056 Basel, Switzerland*

Received 1 November 2005; received in revised form 4 March 2006; accepted 6 March 2006
Available online 2 May 2006

Abstract

The goal of this study was to investigate the potential for atmospheric Hg° uptake by grassland species as a function of different air and soil Hg exposures, and to specifically test how increasing atmospheric CO_2 concentrations may influence foliar Hg concentrations. Four common tallgrass prairie species were germinated and grown for 7 months in environmentally controlled chambers using two different atmospheric elemental mercury (Hg°; 3.7 ± 2.0 and 10.2 ± 3.5 ng m^{-3}), soil Hg (<0.01 and 0.15 ± 0.08 μg g^{-1}), and atmospheric carbon dioxide (CO_2) (390 ± 18, 598 ± 22 μmol mol^{-1}) exposures. Species used included two C4 grasses and two C3 forbs. Elevated CO_2 concentrations led to lower foliar Hg concentrations in plants exposed to low (i.e., ambient) air Hg° concentrations, but no CO_2 effect was apparent at higher air Hg° exposure. The observed CO_2 effect suggests that leaf Hg uptake might be controlled by leaf physiological processes such as stomatal conductance which is typically reduced under elevated CO_2. Foliar tissue exposed to elevated air Hg° concentrations had higher concentrations than those exposed to low air Hg°, but only when also exposed to elevated CO_2. The relationships for foliar Hg concentrations at different atmospheric CO_2 and Hg° exposures indicate that these species may have a limited capacity for Hg storage; at ambient CO_2 concentrations all Hg absorption sites in leaves may have been saturated while at elevated CO_2 when stomatal conductance was reduced saturation may have been reached only at higher concentrations of atmospheric Hg°. Foliar Hg concentrations were not correlated to soil Hg exposures, except for one of the four species (Rudbeckia hirta). Higher soil Hg concentrations resulted in high root Hg concentrations and considerably increased the percentage of total plant Hg allocated to roots. The large shifts in Hg allocation patterns—notably under soil conditions only slightly above natural background levels—indicate a potentially strong role of plants in belowground Hg transformation and cycling processes.

Keywords: Mercury; Foliar uptake; Tallgrass prairie; Bioaccumulation

1. Introduction

The predominant mercury (Hg) species (~95%) in the atmosphere is elemental mercury (Hg°) (Fitzgerald et al., 1991), with the remaining ~5% consisting of reactive gaseous mercury and particulate Hg (Schroeder and Munthe, 1998). Current global anthropogenic Hg emissions to the atmosphere are thought to be three times pre-industrial anthropogenic emissions (Seigneur et al., 2004). Recent work has shown that plant foliage can be a significant sink for atmospheric Hg° (Hanson et al., 1995; Ericksen et al., 2003; Frescholtz et al., 2003; Schwesig and Krebs, 2003) and that litterfall can be an important source of Hg to the terrestrial ecosystem (Frescholtz et al., 2003). Foliar Hg concentrations can vary widely as a function of species type (Rasmussen, 1995; Schwesig and Krebs, 2003). Frescholtz et al. (2003) showed that aspen foliage accumulated Hg over time and that leaf Hg concentrations were predominantly controlled by atmosphere Hg° concentrations

* Corresponding author. Tel.: +1 775 784 4203; fax: +1 775 784 4789.
E-mail address: msg@unr.nevada.edu (M.S. Gustin).

0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.03.008

with a minor influence of soil Hg concentrations. Three potential pathways for Hg movement into foliage have been suggested: (1) cuticular uptake of Hg either from dry or wet deposition to the leaf surface (Lindberg et al., 1992, 1994); (2) direct uptake of gaseous Hg° through stomata (Browne and Fang, 1978; Mosbaek et al., 1988; Ericksen et al., 2003; Schwesig and Krebs, 2003); and (3) uptake from the soil and translocation to the leaf via the transpiration stream (Bishop et al., 1998; Leonard et al., 1998).

The present atmospheric carbon dioxide (CO_2) concentration is the highest recorded in the last 420 000 years (Petit et al., 1999) (~360 μmol mol^{-1}) and a rapid concentration increase has occurred in the last 120 years (Intergovernmental Panel on Climate Change, 2001). This ongoing change has the potential to impact accumulation and turnover of Hg by plants since plant responses to elevated CO_2 can potentially affect all three pathways of Hg uptake. For example, elevated CO_2 exposures decrease stomatal conductance and abundance (Knapp et al., 1993; Owensby et al., 1997) and have been found to alter biomass production and species composition (Owensby et al., 1999; Zavaleta et al., 2003). To our knowledge, there have been no studies to date that looked at the influence of atmospheric CO_2 concentrations on foliar uptake of atmospheric Hg°.

The goal of this study was to investigate atmospheric Hg° uptake by grassland species as a function of different air and soil Hg exposures, and to specifically test how increasing atmospheric CO_2 concentrations may affect this. The study used two grasses and two forbs common in tall grass prairie ecosystems. The plant species have physiological differences and different growth patterns. In addition, the two forbs, *Chamaecrista nictitans* (partridge pea) and *Rudbeckia hirta* (blackeyed Susan) use a different photosynthetic pathway (i.e. C3 pathway) compared to the two grasses *Andropogon gerardii* (big bluestem grass) and *Sorghastrum nutans* (Indiangrass) (C4 species). C3 species generally show stronger responses to elevated CO_2 when compared to C4 species, which are CO_2-saturated already at ambient CO_2 conditions (Hopkins, 1999). The research hypotheses for this project were: (1) elevated atmospheric CO_2 concentrations would result in reduced foliar Hg concentrations due to reduced stomatal conductance; (2) elevated CO_2 concentrations would have a greater effect on leaf Hg concentrations for C3 species than C4 species, (3) foliar Hg concentrations would be influenced primarily by air Hg° concentrations, and (4) root Hg concentrations would be influenced primarily by soil Hg concentrations.

2. Materials and methods

The experiment was conducted using environmentally controlled, naturally lit plant growth chambers or ecopods, located in the Frits Went Greenhouse at the Desert Research Institute (Reno, Nevada, USA). Ecopods (dimensions: 1.14 m × 0.99 m × 1.48 m) are totally enclosed with glass on all four sides and the top. Air temperatures inside ecopods were maintained at 14.8 ± 0.4 °C during nighttime hours and 24.8 ± 0.7 °C during daytime hours, with day and night hours based on incident light measured by a photon-flux sensor (Li-Cor, Lincoln, NE, USA). Each of the eight ecopods housed 16 plant pots made of polyvinyl chloride pipe (diameter, 15 cm; length, 46 cm) with a pipe cap at base. Plants were grouped according to species in the ecopods. Air was well mixed in each pod.

The experiment used a split-plot design with air Hg° and CO_2 concentrations as main factors and soil Hg concentrations and plant species nested within air manipulations (Fig. 1). Atmospheric exposures in the ecopods were

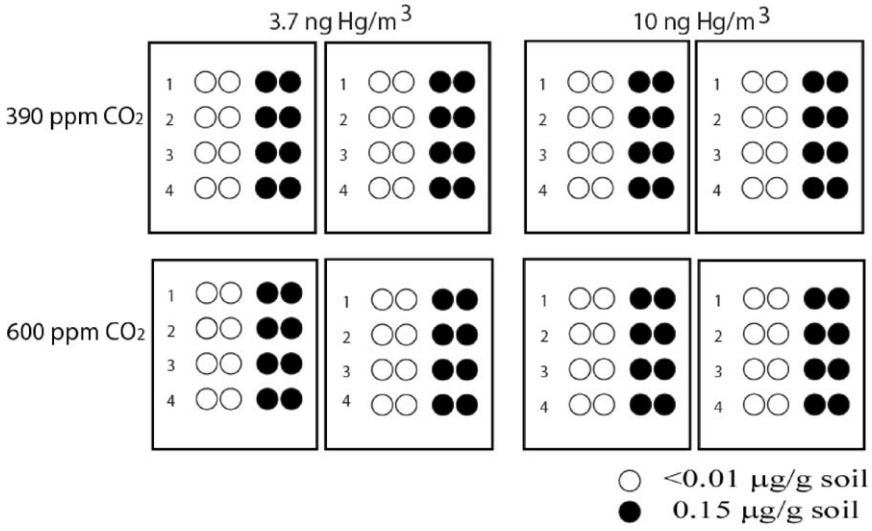

Fig. 1. Schematic showing the experimental design for the project. Split-plot design with air Hg° and CO_2 concentrations as main factors. Soil Hg concentration and plant species were nested treatments within air manipulations. Each ecopod housed a total of 16 pots with two soil Hg concentration exposures (eight pots each), and four tallgrass prairie species. Circles within each ecopod represent pots, each of which contained one of the four species (rows: 1 = *A. gerardii*; 2 = *S. nutans*; 3 = *C. nictitans*; 4 = *R. hirta*).

applied in a full factorial design in two replicates with two CO_2 concentrations (ambient greenhouse: 390 ± 18 μmol mol^{-1}; elevated 598 ± 22 μmol mol^{-1}) and two air Hg° concentrations (ambient greenhouse: 3.7 ± 2.0 ng m^{-3}; elevated: 10.2 ± 3.5 ng m^{-3}) (Fig. 1). Carbon dioxide exposures were initiated at the start of the experiment and atmospheric Hg° exposures were started once seeds had germinated at week seven. Gaseous Hg° for the elevated air Hg° exposure ecopods was produced using permeation tubes (VICI Metronics, Poulsbo, Washington, USA) contained in glass housing and submerged in a constant-temperature (38 °C) water bath. The amended air was introduced to ecopod air supply at a controlled rate using a mass flow controller. Total gaseous Hg° concentrations were monitored using a Tekran™ 2357 A Hg analyzer (Toronto, ON, Canada) and a computer-controlled six-valve solenoid switching unit that allowed for five minute sampling in each ecopod. The Tekran™ 2357A analyzer is thought to measure predominantly Hg° (Lindberg et al., 2002a,b). Carbon dioxide was added using a gas cylinder (100% CO_2) with flows controlled automatically to maintain programmed concentrations (Verburg et al., 2004). Carbon dioxide concentrations were monitored using a Li-Cor 6252 (Lincoln, NE, USA) analyzer and a solenoid switching unit. Data were averaged over 5 min intervals and recorded using a data logger (Model 10X, Campbell Scientific Inc., Logan, USA).

Soil was collected from a grassland field site at the Kessler Farm Field Laboratory near Norman, Oklahoma, USA. Half of all plants were grown in unamended soil (<0.01 μg Hg g^{-1}) which is further referred to as background soil Hg concentrations. The remaining plants were grown in this same soil amended with mercury chloride ($HgCl_2$) to obtain a higher soil Hg concentration of 0.15 ± 0.08 (standard deviation) μg g^{-1}. Mercury chloride was chosen as the amendment form because it is thought to be one of the Hg species entering terrestrial ecosystems from precipitation (Schroeder and Munthe, 1998). This chemical form was easy to obtain and add to the soils and is water soluble making it available to plants in soil solution. Total Hg concentrations in soil were determined by the Nevada Bureau of Mines and Geology using cold vapor atomic spectrometry after digestion in *aqua regia* (Lechler, 1999).

Approximately 20 seeds (Ion Exchange Seed Company, Harpers Ferry, Iowa, USA) of one of the four species were sown (128 total pots; 32 pots per species), germinated and grown in each pot. The pots were watered twice daily with greenhouse tap water (3.9 ± 1.1 ng Hg l^{-1}) for the first two months, then once a day for the following month, and then every other day for the last four months. Water content in soil was checked biweekly to maintain approximately 15% water content based on weight. Total Hg concentrations in irrigation samples were determined bimonthly by oxidation of the sample with 0.1 M bromine monochloride followed by stannous chloride reduction with the Hg° then purged onto gold-coated silica sand traps using pre-purified N_2 gas. Mercury on traps was quantified using dual amalgamation and cold-vapor atomic fluorescence spectrometry (Bloom and Fitzgerald, 1988).

The first foliage harvest was done one week after plant germination, once sufficient plant tissue was available, and then followed by four monthly harvests of one whole plant from each pot. Plant shoots were cut just above the soil surface using a clean razor blade and placed on a Kim Wipe®. Harvested plants were then rinsed by submerging each plant, using Teflon®-coated tweezers, in two large acid-cleaned glass Petri® dishes filled with ~100 ml of 18.2 MΩ/cm deionized water. New water was used for each plant. Rinsed plant material was then placed in Ziploc® sandwich bags, so as to show the entire plant leaf area, and bags were photocopied and scanned to determine leaf areas using an imaging software program (Image Pro-Plus 4.5.1.22. Media Cybernetics, Silver Spring, MD, USA).

Plant material was frozen with liquid nitrogen and homogenized using acid-cleaned mortar and pestles and placed in vials (Fisher Scientific, Fair Lawn, NJ, USA) within one day after harvesting. Filled pre-weighed vials were lyophilized for 48 h (Virtis Benchtop 3L, Gardiner, NY, USA), the dry weight of plant tissue recorded, and then stored at −20 °C until analyses. Liquid nitrogen and lyophilization have been reported to have no effect on Hg concentrations (Kolka et al., 1999; Ericksen et al., 2003). Plant samples were analyzed for Hg content using a Milestone™ DMA 80 (Monroe, CT, USA; EPA method 7473), which determines total Hg by thermal decomposition, amalgamation, and atomic absorption spectrometry. Daily calibration checks were performed using at least three certified reference materials (National Institute of Standards and Technology, Gaithersburg, MD, USA; NIST standard reference material #1515, #1547, #1573a, and #2709). Error for standards was less than 5% and triplicate analyses of every tenth sample analyzed showed minimal sample variance (aboveground tissue, $n = 50$, coefficient of variance $= 8.4 \pm 7.2\%$; belowground tissue, $n = 13$, coefficient of variance $= 29.2 \pm 19.2\%$). All data presented for tissue Hg concentrations are a function of dry weight.

At the end of the experiment, all aboveground biomass was sequentially harvested between the 27th and the 29th weeks of the experiment. Leaves, stems, and petioles were separated and each plant component was washed and placed in pre-weighed vials. The washing procedure for all aboveground plant tissue was the same as described above. Plant material was then lyophilized and total dry mass and Hg concentration of each component determined. The roots of all four species were separately harvested the 29–32nd weeks of the experiment. Small sub-samples of washed root material from each pot were placed in vials, lyophilized and then analyzed for total Hg. The remaining root material was dried for 48 h at 55 °C and weighed. Before Hg analysis, all above and belowground materials were homogenized in a small stainless steel grinder. Leaf areas for vegetation were calculated

using an allometric relationship between leaf area (LI-3100 leaf area meter, LI-COR, Lincoln, NE) and dry weight using subsets from each treatment ($n = 48$).

ANOVA analyses of the dependent variable leaf mercury concentration were done using all harvest data with the exception of the first (see Section 3) using the independent variables CO_2 ($n = 2$; ambient CO_2: 390 μmol mol^{-1} and elevated CO_2: 598 μmol mol^{-1}), air Hg° concentrations ($Hg°_{air}$; $n = 2$; ambient air: 3.7 ng m^{-3} and elevated: 10.2 ng m^{-3}), soil Hg concentration (Hg_{soil}; $n = 2$: low soil Hg: <0.01 μg g^{-1} and high soil Hg: 0.15 μg g^{-1}), species ($n = 4$), and time ($n = 3$ harvests) using STATA® 9 software. Variables were treated as categorical variables with the exception of the harvest time which was treated as a continuous variable. Then, separate ANOVAs were performed for plants exposed to ambient and elevated air Hg° exposures (Table 2b) and plants exposed to ambient and elevated CO_2 (Table 2c), respectively. Using data for only the final harvest, the statistical significance of air and soil Hg exposures, CO_2 concentration manipulations and plant species on foliar and root Hg concentrations were tested with ANOVAs using Number Cruncher Statis-

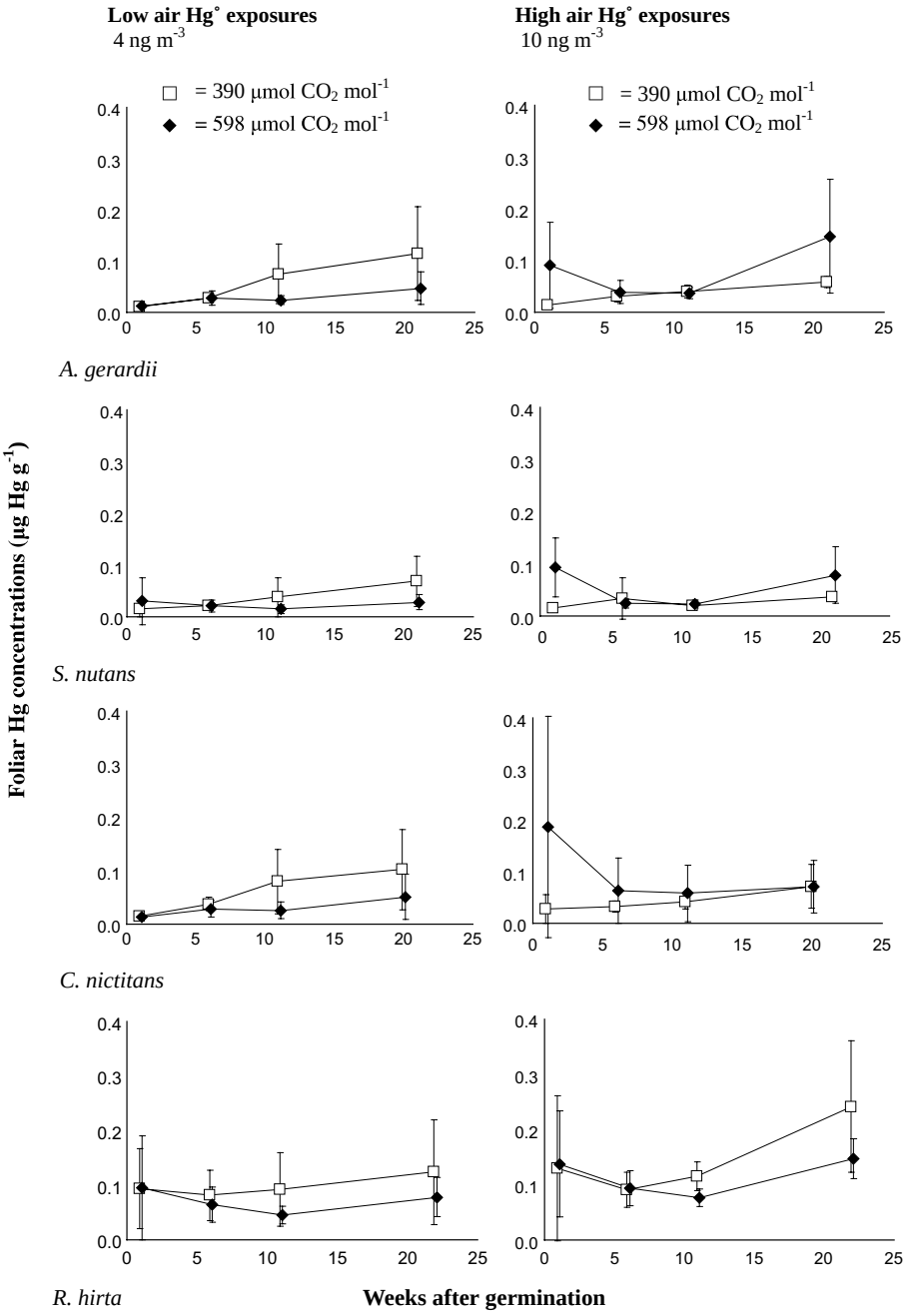

Fig. 2. Mean of all foliar Hg concentrations (±standard deviation) over time as a function of atmospheric CO_2 concentration at low and high air Hg° concentrations, with soil Hg exposures combined. Points are slightly horizontally offset to help differentiate between error bars.

tical Package© (NCSS) 2004. Relationships were considered statistically significant when $p < 0.05$.

3. Results

Tissue Hg concentrations for similar exposures in replicate pods were not statistically significantly different. The good reproduction of foliar Hg concentrations between replicate pods has been seen in other studies using the same experimental design (Frescholtz et al., 2003; Millhollen et al., submitted for publication. A significant effect of soil Hg concentrations was found based on ANOVA analyses using foliar tissue concentrations from all harvests except the first ($P < 0.001$; Table 2). This result however, was based on the statistical effect of one species (*R. hirta*) only, and the effect disappeared when *R. hirta* was removed from the analysis. Thus, in Fig. 2, tissue Hg concentrations for the two soil Hg exposures were combined in order to clarify effects of air CO_2 and air Hg° exposures on foliar Hg concentrations.

For some species, the highest foliar concentrations and the most variable concentrations were observed at the first harvest (more often at the high CO_2 and high Hg° air exposures). Because of this and other reasons described below, the data from the first harvest which immediately followed plant germination were not used for analyses investigating the effect of air Hg° and air CO_2 concentrations on foliar Hg concentrations. Using data from the last three harvest

Table 1
Mean Hg concentration and standard deviation in aboveground plant parts at the final harvest ($t = 30$–32 weeks) as a function of air Hg and CO_2 concentrations and soil Hg concentrations (low = <0.01 μg g^{-1}; high = 0.15 μg g^{-1})

Species	390 ppm CO_2						600 ppm CO_2					
	Soil Hg	Hg concentration (μg g^{-1})	*n*	Soil Hg	Hg concentration (μg g^{-1})	*n*	Soil Hg	Hg concentration (μg g^{-1})	*n*	Soil Hg	Hg concentration (μg g^{-1})	*n*
Air 4 ng Hg m^{-3}												
A. gerardii	Low	0.12 ± 0.09	4	High	0.14 ± 0.11	4	Low	0.05 ± 0.04	4	High	0.04 ± 0.03	4
S. nutans	Low	0.07 ± 0.05	4	High	0.07 ± 0.05	4	Low	0.02 ± 0.01	4	High	0.03 ± 0.02	4
R. hirta	Low	0.09 ± 0.09	4	High	0.19 ± 0.17	4	Low	0.05 ± 0.03 a	4	High	0.11 ± 0.07 ac	4
C. nictitans	Low	0.10 ± 0.11	4	High	0.10 ± 0.13	4	Low	0.05 ± 0.04	3	High	0.03 ± 0.02	4
Air 10 ng Hg m^{-3}												
A. gerardii	Low	0.06 ± 0.03	4	High	0.05 ± 0.02	4	Low	0.13 ± 0.11	4	High	0.19 ± 0.11	4
S. nutans	Low	0.03 ± 0.01	4	High	0.04 ± 0.02	4	Low	0.08 ± 0.06	4	High	0.08 ± 0.06	4
R. hirta	Low	0.21 ± 0.11	4	High	0.29 ± 0.14	3	Low	0.09 ± 0.03 b	4	High	0.21 ± 0.03 bc	4
C. nictitans	Low	0.06 ± 0.06	4	High	0.10 ± 0.09	4	Low	0.08 ± 0.08	4	High	0.08 ± 0.07	4

Letters denote the comparisons that were statistically significant different.

Table 2
P values for ANOVA Analyses using all foliar Hg concentrations collected after the first harvest

Source	df	MS	*P*
Panel a: ANOVA of all treatments			
CO_2	1	0.020	*0.004*
Hg_{air}	1	0.023	*0.002*
Species	3	0.083	*<0.001*
Hg_{soil}	1	0.027	*<0.001*
Time	1	0.118	*<0.001*
Residual	372	0.002	
Total	379	0.004	
Panel b: Separate ANOVAs for plants exposed to Hg_{air} ambient and Hg_{air} elevated (bold letters)			
CO_2	1	0.055/**0.001**	<0.001/***0.523***
Species	3	0.020/**0.072**	*<0.001/* < ***0.001***
Hg_{soil}	1	0.010/**0.018**	*0.023*/***0.009***
Time	1	0.036/**0.088**	<0.001/ < ***0.001***
Residual	184/**182**	0.002/**0.003**	
Total	190/**188**	0.003/**0.004**	
Panel c: Separate ANOVAs for plants exposed to CO_2 ambient and CO_2 elevated (bold letters)			
Hg_{air}	1	0.001/**0.585**	*0.603/* < ***0.001***
Species	3	0.065/**0.024**	<0.001/ < ***0.001***
Hg_{soil}	1	0.015/**0.012**	*0.021*/***0.009***
Time	1	0.089/**0.035**	<0.001/ < ***0.001***
Residual	182/**184**	0.003/**0.002**	
Total	188/**190**	0.004/**0.003**	

dates foliar Hg concentrations of the four species were significantly lower under elevated CO_2 compared to plants exposed to ambient CO_2 concentrations (Fig. 2; Table 2). This CO_2 effect, however, was only observed when plants were also exposed to ambient Hg° air concentrations (i.e., 4 ng m^{-3}; Table 2b). The observed CO_2 effects observed at ambient Hg° exposure became apparent at the second harvest (i.e., six weeks after germination) and the mean concentration differences increased during the experiment. No statistically significant CO_2 effect was observed when plants were exposed to higher air Hg° concentrations (i.e., 10 ng m^{-3}). When analyzed at the level of individual species using data from the last harvest, statistical significance of the CO_2 exposure influence on foliar Hg concentrations was only found for *R. hirta*. Although this data suggests differences among the four species, the large variability of measured Hg concentrations resulted in a lack of the statistical power necessary to properly assess species-specific responses or the behavior of different functional groups (e.g., C3 plants versus C4 plants) at the replications implemented in this study. Elevated CO_2 concentrations did not significantly increase aboveground biomass and leaf areas of any of the species. Effects of air Hg° exposure on foliar Hg concentrations differed between the two CO_2 treatments (Fig. 2, Table 2c). At ambient CO_2 concentrations, no differences in foliar tissue Hg concentrations were observed between the two air Hg° exposures, but at elevated CO_2 foliar Hg concentrations were significantly greater at the higher atmospheric Hg° concentrations (Fig. 2; Tables 1 and 2c).

From harvest two to harvest four, there was a significant increase in foliar Hg concentration (Fig. 2; Table 2), although this effect disappeared when data of the first harvest (i.e., immediately after germination) was included. The significance of time from harvest two to harvest four was supported by linear regression analyses (data not shown) that indicated weak but significant correlations of Hg foliar tissue concentration with time for all species (coefficients of variability, r^2, ranged from 0.05 to 0.2 for the four species).

Mercury concentrations in root tissue of all four species at the final harvest were significantly greater for plants grown in high Hg soil versus low Hg soil concentration exposures (Fig. 3) with concentrations up to 100 times higher. Root Hg concentrations, however, were not influenced by air Hg° and CO_2 exposures. Root Hg concentrations were similar among the four species. Atmospheric CO_2 exposures were not found to have resulted in any increased root biomass.

Fig. 4 illustrates the percent total Hg in aboveground and root tissue for the four species as a function of soil Hg concentration exposures. When plants were grown in unamended background soils, a higher percentage of Hg was in the aboveground tissue which contained 50–70% of the total plant mercury. When plants were grown in soils amended with $HgCl_2$, belowground tissue contained the largest percentage of total Hg (74–95%), with aboveground tissue containing a smaller percent (5–26%).

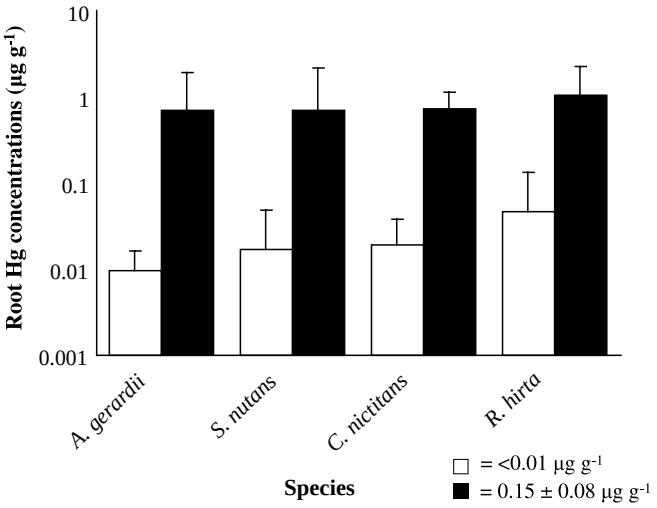

Fig. 3. Mean Hg concentration (±standard deviation) in roots ($t = 29$–32 weeks) for all species as a function of soil concentration exposures, with all air treatments combined.

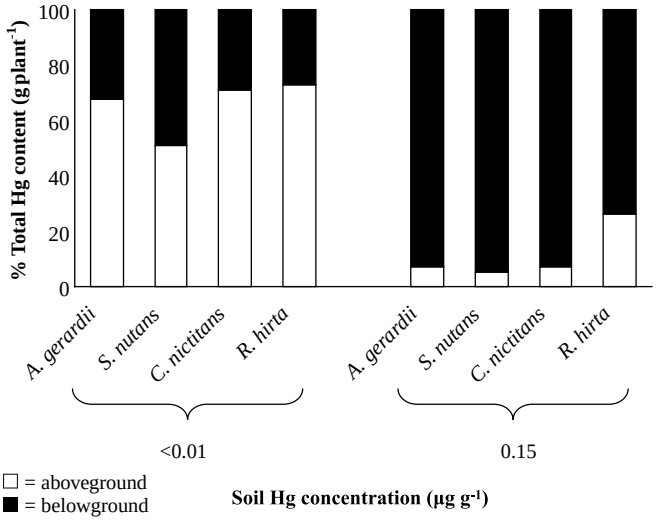

Fig. 4. Percent total Hg content in plant as a function of soil Hg exposures and plant species, with all air Hg° and CO_2 treatments combined.

4. Discussion

The observed reduction in foliar Hg concentrations under elevated atmospheric CO_2 concentrations and low ambient air Hg° exposure supports our first hypothesis that CO_2 can reduce Hg uptake of plants under certain conditions. The lower foliar Hg concentrations at the higher CO_2 exposures may be due to reduced stomatal conductance which has been observed at elevated CO_2 concentrations (30–50%: Woodward et al., 1991). In contrast there were no consistent CO_2 effects on foliar Hg concentrations when plants were grown under elevated air Hg° concentrations. Leaf areas and biomass were not affected by elevated CO_2 or Hg exposures suggesting that changes in plant sur-

face area was not driving the observed lower foliar Hg concentrations at the higher CO_2 exposure.

A possible reason for the occurrence of a CO_2 effect under low Hg° exposure concentrations, and the disappearance of a clear effect at higher Hg° levels, is that reduced stomatal conductance under elevated CO_2 might limit the transport of atmospheric Hg° into leaves, a limitation that disappears when atmospheric Hg° concentrations are high. A very similar interaction between atmospheric Hg° and CO_2 was apparent for the two air Hg° exposures: increased air Hg° concentrations were correlated with increased foliar Hg concentrations only at elevated CO_2 exposures, or in other words when stomatal conductance was low. These apparent interactions between atmospheric CO_2 and Hg° exposure might indicate a maximum capacity of Hg° that can be assimilated or stored in leaves; all absorption sites in leaves might become saturated when air Hg° are high (i.e. under high atmospheric Hg° exposure) or when stomatal conductance is high (i.e. ambient CO_2), but not under ambient atmospheric Hg° levels when stomatal conductance is reduced because of elevated CO_2. The contrasting CO_2 effects as a function of atmospheric Hg° conditions also point towards the air as the source of Hg in leaves as opposed to Hg transport by the transpiration stream, although detailed studies of Hg uptake and allocation patterns in plant leaves are necessary to confirm this.

This study did not support the second hypothesis that CO_2 exposures would have greater influence on Hg uptake for C3 species compared to C4 species. Although figures and ANOVAs indicate the presence of some species differences, the statistical power to detect species-specific responses or to assess responses of functional groups (i.e., C3 versus C4 plants) was not high enough against the large variability in observed foliage Hg concentrations. Due to the low exposure treatments selected in our study as compared to some other studies (e.g., high air Hg° concentrations of 10 ng m^{-3} compared to 70 ng m^{-3} [Frescholtz et al., 2003] or 30 ng m^{-3} [Ericksen and Gustin, 2004]) the differences between treatments were generally small and difficult to detect against the large background variability. The data from these low exposure treatments yielded results of ecological significance for the range of soil and air treatments utilized are similar to levels observed in natural settings, while most previous controlled experimental work has been done at higher exposure concentrations.

If all species are lumped together foliar Hg concentrations were significantly different as a function of soil Hg exposures (Table 2); however, this result was purely driven by *R. hirta* (discussed in detail below). None of the other three species showed a statistical relationship between foliar Hg concentrations and soil Hg concentrations. The observed interactions of CO_2 and air Hg concentrations, along with the fact that soil Hg concentration only influenced foliar Hg concentration in one of the four species, supports the third hypothesis that air Hg concentrations are more important in influencing foliar Hg concentrations than soil Hg concentrations. These results are in agreement with results from Frescholtz et al. (2003) which indicated that aspen leaf Hg concentrations were predominantly controlled by atmosphere Hg° concentrations with soil Hg concentrations exerting a minor influence.

The foliar concentrations of the same grassland species grown in situ at the Kessler experimental farm near Norman, Oklahoma and in a mesocosm study (Obrist et al., 2005), exposed to the same soil substrate (<0.01 μg Hg g^{-1}) but to lower atmospheric Hg° concentrations (1.6–2 ng m^{-3}), were much lower (~0.002–0.02 μg g^{-1}; Stamenkovic and Gustin, personal communication) as compared to this study. Possibly, the increase from the global ambient background levels (~1.6 ng m^{-3}; Schroeder and Munthe, 1998; Lamborg et al., 2002) to the indoor concentrations observed in this greenhouse study (e.g. 4 ng m^{-3} in the present study) might have a larger effect on foliar Hg levels than further increases in atmospheric Hg° applied in our study (e.g., from 4 to 10 ng m^{-3}). However, other possible reasons for the difference in field and laboratory derived data are many, including different age of plants or the use of undisturbed versus disturbed soils. The lower foliar concentrations observed in the field as compared to this greenhouse study, however, raises important questions about the applicability and extrapolation of greenhouse study results to the field.

As mentioned above, if foliar Hg concentrations are compared as a function of soil exposures, the only species with a significant difference is *R. hirta*. Compared to the three other species, *R. hirta* has large basal leaves which are covered by short coarse hairs that grow close to the soil surface. Since *R. hirta* has leaves closer to the soil surface than the other species studied, emission of soil Hg and subsequent uptake by the basal leaves or contamination of leaves by soil substrate may have influenced foliar Hg concentrations. Similar to our study, Ericksen et al. (2003) found no variation in leaf Hg concentrations between soil Hg exposures, but Frescholtz et al. (2003) found that soil Hg concentrations significantly influenced aspen leaf Hg concentration. However, soil concentrations applied in their study were much higher than in this study, and statistical analyses overall indicated a more important influence of air Hg° concentrations on foliar Hg° concentrations when compared to soil exposure concentrations.

ANOVA analyses comparing foliar Hg concentrations as a function of time for all data collected after the first harvest indicate a significant increase in concentrations as a function of time, and regression analyses for each species support these time trends although the correlation coefficients were low. Foliar Hg concentrations observed at the first harvest, which occurred only a week after germination, were most variable and in some cases greater than the following harvest. The high and variable tissue Hg concentrations measured in this initial harvest may have been due to leaves being very close to the soil surface resulting in Hg emitted from the soils being adsorbed by plant tissue. However, it is noteworthy that the high initial harvest Hg

concentrations were most often associated with the high CO_2 and high Hg exposures. This may indicate that there was some physiological or morphological effect of the exposure conditions initially on the plants that influenced air Hg uptake. It is important to mention that the foliar Hg data presented in our study are concentrations and do not represent total Hg uptake by plant foliage. Time trends of total foliar Hg accumulation on a biomass basis would be more pronounced than the presented Hg concentrations on a dry weight basis. Because leaf biomass and leaf area were not affected by air Hg and air CO_2 concentrations, however, the reported CO_2 and air Hg effects are also valid for total Hg uptake of plants.

Root tissue Hg concentrations at the final harvest increased with increasing soil Hg concentrations for all four plant species. This result is consistent with other studies (Lindberg et al., 1979; Cocking et al., 1995; Ericksen et al., 2003; Frescholtz et al., 2003); however, the current study was different from others in that soil Hg exposure concentrations were significantly lower in magnitude and range, and similar to that expected in much of the global terrestrial landscape (Connor and Shacklette, 1975; Zhang et al., 2001; Ericksen et al., in press). It was striking that an increase of soil concentrations from <0.01 to $0.15\ \mu g\ g^{-1}$, with the latter only slightly above global natural background, led to a shift of Hg being allocated mainly in aboveground compartments (50–70% of total plant Hg) to predominately being allocated in roots (74–95%). This shift in Hg allocation from aboveground to belowground tissues indicates a potentially strong role of plants in belowground Hg transformation and cycling processes.

Acknowledgements

Funding for this project was provided by the National Science Foundation, Atmospheric Sciences Division grant (0214765), and the Environmental Protection Agency, STAR grant (R_82980001_0). The research was supported by NSF and EPA, but the opinions presented herein are not representative of these agencies. We thank M. Markee, R. Bergin, L. Martindale, B. Hachett and T. Dominguez for their countless hours of help with sample collection, processing and data analysis. Special thanks to J. Arnone, D. Johnson and G. Miller for input as thesis committee members and D. Schorran for the technical assistance at the Desert Research Institute.

References

Bishop, K.H., Lee, Y.H., Munthe, J., Dambrine, E., 1998. Xylem sap as a pathway for total mercury and methylmercury transport from soils to tree canopy in the boreal forest. Biogeochemistry 40, 101–113.

Bloom, N.S., Fitzgerald, W.F., 1988. Determination of volatile mercury species at the pictogram concentration by low temperature gas chromatography with cold-vapor atomic fluorescence determination. Anal. Chim. Acta 208, 151–161.

Browne, C.L., Fang, S.C., 1978. Uptake of mercury vapor by wheat. Physiol. Plant 61, 430–433.

Cocking, D., Rohrer, M., Thomas, R., Walker, J., Ward, D., 1995. Effects of root morphology and mercury concentration in the soil on uptake by terrestrial vascular plants. Water Air Soil Pollut. 80, 1113–1116.

Connor, J.J., Shacklette, H.T., 1975. Background geochemistry of some rocks, soils, plants, and vegetables in the conterminous United States. US Geological Survey Professional Paper 574-F, p. 168.

Ericksen, J.A., Gustin, M.S., 2004. Foliar exchange of mercury as a function of soil and air concentration. Sci. Tot. Env. 324, 271–279.

Ericksen, J.A., Gustin, M.S., Schorran, D.E., Johnson, D.W., Lindberg, S.E., Coleman, J.S., 2003. Accumulation of atmospheric mercury in forest foliage. Atmos. Environ. 37, 1613–1622.

Ericksen, J.A., Gustin, M.S., Xin, M., Weisberg, P.J., Fernandez, G.C.J., in press. Air–soil exchange of mercury from background soils in the United States. Sci. Tot. Environ.

Fitzgerald, W.F., Mason, R.P., Vandal, G.M., 1991. Atmospheric cycling and air–water exchange of mercury over mid-continental lacustrine regions. Water Air Soil Pollut. 56, 745–767.

Frescholtz, T.F., Gustin, M.S., Schorran, D.E., Fernandez, G.C.J., 2003. Assessing the source of mercury in the foliar tissue of Quaking Aspen. Environ. Toxicol. Chem. 22, 2114–2119.

Hanson, P.J., Lindberg, S.E., Tabberer, T.A., Owens, J.G., Kim, K.H., 1995. Foliar exchange of mercury vapor: evidence for a compensation point. Water Air Soil Pollut. 80, 373–382.

Hopkins, W.G., 1999. Introduction to Plant Physiology. John Wiley, New York.

Intergovernmental Panel on Climate Change, 2001. Working Group 1, Third Assessment Report. Cambridge University Press, Cambridge, UK.

Knapp, A.K., Hamerlynck, E.P., Owensby, C.E., 1993. Photosynthetic and water relations responses to elevated CO_2 in the C4 grass *Andropogon gerardii*. Int. J. Plant Sci. 154, 459–466.

Kolka, R.K., Nater, E.A., Grigal, D.F., Verry, E.S., 1999. Atmospheric inputs of mercury and organic carbon into a forested upland/bog watershed. Water Air Soil Pollut. 113, 273–294.

Lamborg, C.H., Fitzgerald, W.F., O'Donnell, J., Torgersen, T., 2002. A non-steady-state compartmental model of global-scale mercury biogeochemistry with interhemispheric atmospheric gradients. Geochim. Cosmochim. Acta 66, 1105–1118.

Lechler, P.J., 1999. Modern mercury contamination from historic amalgamation milling of silver ores in the Carson River, Nevada and Jordan Creek, Idaho: importance of speciation analysis in understanding the source, mobility, and fate of polluted materials. In: Ebinhaus, R., Turner, R.R., de Lacerda, L.D., Vasiliev, O., Salomons, W. (Eds.), Mercury Contaminated Sites: Characterization, Risk Assessment and Remediation. Spinger, Berlin, p. 538.

Leonard, T.L., Taylor, G.E., Gustin, M.S., Fernandez, G.C.J., 1998. Mercury and plants in contaminated soils: 1. Uptake, partitioning, and emission to the atmosphere. Environ. Toxicol. Chem. 17, 2063–2071.

Lindberg, S.E., Jackson, D.R., Huckabee, J.W., Janzen, S.A., Levin, M.J., Lund, J.R., 1979. Atmospheric emissions and plant uptake of mercury from agricultural soils near the Almadén mercury mine. J. Environ. Qual. 8, 572–578.

Lindberg, S.E., Meyers, T.P., Taylor, G.E., Turner, R.R., Schroeder, W.H., 1992. Atmosphere–surface exchange of mercury in a forest: results of modeling and gradient approaches. J. Geophys. Res—Atmos. 97, 2519–2528.

Lindberg, S.E., Owens, J.G., Stratton, W.J., 1994. Application of throughfall methods to estimate dry deposition of mercury. In: Watras, C.J., Huckabee, J.W. (Eds.), Mercury Pollution: Integration and Synthesis. Lewis Publishers, Chelsea, MI, pp. 261–271.

Lindberg, S.E., Dong, W., Meyers, T., 2002a. Transpiration of gaseous elemental mercury through vegetation in a sub-tropical wetland in Florida. Atmos. Environ. 36, 5207–5219.

Lindberg, S.E., Zhang, H., Vette, A.F., Gustin, M.S., Barnett, M.O., Kuiken, T., 2002b. Dynamic flow chamber measurements of gaseous mercury emission fluxes over soils: Part 2—effect of flushing flow rate

and verification of a two-resistance exchange interface simulation model. Atmos. Environ. 36, 847–859.

Millhollen, A.G., Gustin, M.S., Obrist, D., submitted for publication. Foliar mercury accumulation and exchange for three tree species. Atmos. Environ.

Mosbaek, H., Tjell, J.C., Sevel, T., 1988. Plant uptake of airborne mercury in background areas. Chemosphere 17, 1227–1236.

Obrist, D., Gustin, M.S., Arnone, J.A., Johnson, D.W., Schorran, D.E., Verburg, P.S.J., 2005. Measurements of gaseous elemental mercury fluxes over intact tallgrass prairie monoliths during one full year. Atmos. Environ. 39, 957–965.

Owensby, C.E., Ham, J.M., Knapp, A.K., Bremer, D., Auen, L.M., 1997. Water vapour fluxes and their impact under elevated CO_2 in a C4-tallgrass prairie. Glob. Change Biol. 3, 189–195.

Owensby, C.E., Ham, J.M., Knapp, A.K., Auen, L.M., 1999. Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. Glob. Change Biol. 5, 497–506.

Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pépin, L., Ritz, C., Saltzman, E., Stievenard, M., 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399, 429–436.

Rasmussen, P.E., 1995. Temporal variation of mercury in vegetation. Water Air Soil Pollut. 80, 1039–1042.

Schroeder, W.H., Munthe, J., 1998. Atmospheric mercury—an overview. Atmos. Sci. 32, 809–822.

Schwesig, D., Krebs, O., 2003. The role of ground vegetation in the uptake of mercury and methylmercury in a forest ecosystem. Plant. Soil. 253, 445–455.

Seigneur, C., Vijayaraghavan, K., Lohman, K., Karamchandani, P., Scott, C., 2004. Global source attribution for mercury deposition in the United States. Environ. Sci. Technol. 38, 555–569.

Stamenkovic, J., Gustin, M.S., 2005. Personal communication. Department of Natural Resources and Environmental Sciences, University of Nevada, Reno.

Verburg, P.S.J., Cheng, W., Johnson, D.W., Schorran, D.E., 2004. Nonsymbiotic nitrogen fixation in three-year-old Jeffrey pines and the role of elevated CO_2. Can. J. Forest Res. 34, 1979–1984.

Woodward, F.I., Thompson, G.B., McKee, I.F., 1991. The effects of elevated concentrations of carbon dioxide on individual plants, populations, communities and ecosystems. Ann. Bot. London. 67, 23–38.

Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Thomas, B.D., Cleland, E.E., Field, C.B., Mooney, H.A., 2003. Grassland responses to three years of elevated temperature, CO_2, precipitation and N deposition. Ecol. Monogr. 73, 585–604.

Zhang, H., Lindberg, S.E., Marsik, F.J., Keeler, G.J., 2001. Mercury air/surface exchange kinetics of background soils of the Tahquamenon river watershed in the Michigan upper peninsula. Water Air Soil Pollut. 126, 151–169.